Correction of Spray Concentration and Bioassay Cage Penetration Data

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1. REPORT DATE **2012**	2. REPORT TYPE	3. DATES COVERED **00-00-2012 to 00-00-2012**

4. TITLE AND SUBTITLE **Correction of Spray Concentration and Bioassay Cage Penetration Data**	5a. CONTRACT NUMBER
	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **US Department of Agriculture,Agricultural Research Service,2771 F&B Road,College Station,TX,77845**	8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Field trials were conducted to demonstrate the need for correcting sampled spray concentration data for sampler collection efficiencies and estimated spray exposure levels in mosquito bioassays for cage interference effects. A large spray block was targeted with aerial spray treatments of etofenprox in order to create a gradient in both spray concentration and mortality. Spray concentrations were measured using rotary impactors, which were coupled with caged bioassays. Measured spray concentrations were corrected for sampler collection efficiencies, which ranged from 55% to 15%. The corrected spray concentrations were then used to estimate the spray levels inside the bioassay cages. Given the cage type used (Townzen type) and wind speeds occurring during the spray trials (2?4 m/sec) concentrations inside of the bioassay cage ranged from 65% to 68% of that measured within the spray block. Not correcting for the combination of sampler collection efficiency and cage interference, underestimated spray concentration levels inside the cages were 76?90%. Correcting field-measured data allows not only better comparisons between differing studies, but can also provide better estimates of caged insect mortality versus actual spray concentration exposure levels.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**Same as Report (SAR)**	**4**	

SCIENTIFIC NOTE

CORRECTION OF SPRAY CONCENTRATION AND BIOASSAY CAGE PENETRATION DATA[1]

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ABSTRACT. Field trials were conducted to demonstrate the need for correcting sampled spray concentration data for sampler collection efficiencies and estimated spray exposure levels in mosquito bioassays for cage interference effects. A large spray block was targeted with aerial spray treatments of etofenprox in order to create a gradient in both spray concentration and mortality. Spray concentrations were measured using rotary impactors, which were coupled with caged bioassays. Measured spray concentrations were corrected for sampler collection efficiencies, which ranged from 55% to 15%. The corrected spray concentrations were then used to estimate the spray levels inside the bioassay cages. Given the cage type used (Townzen type) and wind speeds occurring during the spray trials (2–4 m/sec), concentrations inside of the bioassay cage ranged from 65% to 68% of that measured within the spray block. Not correcting for the combination of sampler collection efficiency and cage interference, underestimated spray concentration levels inside the cages were 76–90%. Correcting field-measured data allows not only better comparisons between differing studies, but can also provide better estimates of caged insect mortality versus actual spray concentration exposure levels.

KEY WORDS   Sampler efficiency, collection efficiency, field spray

Evaluating efficacy of aerosol insect control treatments relies on accurate measurements of the amount of both spray material applied and insect mortality within a treated area. However, bioassay cages typically used in these evaluations may inhibit spray penetration into the cage (Boobar et al. 1988, Barber et al. 2006, Hoffmann et al. 2008, Fritz et al. 2010). Coupled with this, estimates of droplet size distributions and concentrations are typically measured by a sampling device that has its own set of operational and spray collection characteristics (May and Clifford 1967, Fritz and Hoffmann 2008, Bonds et al. 2009). Field-collected data can be adjusted to account for both the cage and sampler interaction effects. Seeking to collate results of previous studies, field trials were conducted to demonstrate adjustment of data from measured spray concentrations and caged bioassays.

An Air Tractor 402B (Air Tractor, Olney, TX) was outfitted with 2 Micronair AU5000 nozzles (Micron Sprayers Ltd. Bromyard, Herefordshire, United Kingdom) configured to deliver a volume median diameter ($D_{v0.5}$) of 25 μm at a rate of 44.5 ml/ha. Nozzles were operated at 303 kPa with a number 5 restrictor and the blades at position 5. Applications were made at 63 m/sec at a 6-m boom height for a total swath width of 183 m.

Etofenprox (Zenivex E20; Wellmark International, Schaumburg, IL) was selected as the active ingredient and applied at the lowest labeled rate of 1.96 g/ha to insure gradients in spray concentration and mortality across the block. Zenivex E20 (177.4 g etofenprox/liter) was diluted in BVA 13 (BVA Oils, Wixom, MI) at a rate of 1:3 (Zenivex:oil) (44 g of etofenprox/liter spray material). Fluorescent dye (Uvitex OB, Ciba Corporation, Newport, DE) was also added (3.8 g/liter spray solution).

A 4 × 4 grid (61-m spacing) was established in a field of mowed grass near College Station, TX (30°33′52.51″N, 96°26′21.82″W). At each location, a Florida Latham-Bonds (FLB) (Fritz et al. 2011a) rotary sampler with one Teflon®-coated slide (3 mm width by 6.2 cm height; BioQuip Products, Rancho Dominguez, CA) for droplet sizing and one uncoated slide for deposition analysis were deployed. Mosquito mortality was monitored using Townzen type bioassay cages (Townzen and Natvig 1973) (16 cm diam × 4 cm depth; with T-310 Tulle). Colony-reared *Culex quinquefasciatus* Say and *Aedes albopictus* (Skuse) were used, with 1 cage of each per location. Twenty-five mosquitos were aspirated into each cage.

Prior to each application, cages and samplers were positioned at each location. All cages were

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positioned to face perpendicular into the mean wind direction. During this setup time (approximately 15 min), 2 cages of each species as well as an FLB sampler were deployed as controls. Spray applications were made 91 m upwind of the sample grid. The spray cloud was allowed to drift through the block for 10 min prior to sample collection. Exposed cages were collected into clean plastic bags and stored in an air-conditioned vehicle outside of the spray area. Cages were typically stored for 60–90 min, likely resulting in increased mortality due to residual tarsal contact (Bonds et al. 2010), but this would have been consistent for all treatments. The objective was not evaluation of space spray efficacy, but rather to present a method to correct field-collected data. The uncoated slides were collected individually into labeled bags and the Teflon slides were secured in a labeled tray.

Spray applications were made each morning after wind speed increased to approximately 2 m/sec. Although not typical of most vector control applications, it ensured spray movement through the block with a gradient in both concentration and mortality. Six replicate applications were made across 3 days with the flight line oriented to carry spray through the sampling grid.

One-minute averages of wind speed and direction (R. M. Young model 05701 Wind Monitor-RE; R. M. Young Company, Traverse City, MI), temperature at 5 m (R. M. Young model 43347VC temperature probes in a model 43408 aspirated radiation shield, R. M. Young Company) and RH (R. M. Young model 71372, R. M. Young Company) were measured at a station 30 m downwind of the block.

After exposure, mosquitoes were aspirated into holding cups and mortality counts were made 24 h after treatment. Mosquitoes were considered dead if unresponsive to gentle prodding. Overall insect mortality (M) was calculated from the observed mortality in each cage (MO) and control mortality (MC) via M = [(MO − MC)/(100 − MC)] × 100 (Abbott 1925). Treatments with control mortality over 5% were discarded.

Teflon-coated slides were analyzed using Drop-Vision™ (Leading Edge Associates, LLC, Waynesville, NC). Low spray concentrations through the sampling grid resulted in a minimal numbers of droplets collected (typically 50 or less), requiring searching across slides for droplets and heavily biasing coverage (drops per area) data. Tenth, 50th, and 90th percentile volume diameters ($D_{V0.1}$, $D_{V0.5}$, and $D_{V0.9}$) were recorded.

Deposition-collection slides were processed for mass of tracer dye on the slide, which was then divided by the rotary sampling window area (6.2-cm-tall slides separated by 18.5 cm is 114.7 cm²) (Fritz et al. 2011b). Using the dye mixing rate, the values were converted to volume of etofenprox per vertical area. These data were then corrected for sampling inefficiencies (Fritz et al. 2011a). To effectively use the reported results for multiple wind speeds, data were fit to a quadratic model (CurveExpert; Version 1.4, Daniel Hyams, Hixson, TN) (Eq. 1).

$$\text{Collection efficiency}(\%) = 21.54$$
$$- 2.87 \times \text{wind speed}(\text{m/sec}) \qquad (1)$$
$$- 0.194 \times \text{wind speed}^2$$

The corrected data were then used to estimate the spray material penetrating into the cage using data developed by Fritz et al. (2010) relating internal cage spray concentration to external concentration. The correction factor, A, was fit to a logistic model as function of ambient wind speed (Eq. 2), which is then used to calculate spray penetrating into the cage (Eq. 3).

$$A = \frac{0.67}{1 + 0.84 \times e^{-1.52 \times \text{wind speed}(\text{m/sec})}} \qquad (2)$$

$$\text{Concentration}_{\text{inside cage}} = A \times \text{concentration}_{\text{outside cage}} \qquad (3)$$

Wind speeds ranged from 1.9 to 2.1 m/sec for the 1st 4 treatments, but increased to 4.1 to 4.6 m/sec for the 5th and 6th replications. Droplet size data, although consistent between treatments, were biased due to the small numbers collected. Average $D_{V0.1}$, $D_{V0.5}$, and $D_{V0.9}$ across all treatments were 19, 38, and 55 μm, respectively.

As an example of the correction process, given a sampler-measured spray concentration of 0.00050 μl/cm² of etofenprox and an ambient wind speed of 2.1 m/sec, sampler collection efficiency is 14.6% (Eq. 1), which divided into the 0.00050 μl/cm² results in a corrected etofenprox concentration of 0.00342 μl/cm², which is concentration$_{\text{outside cage}}$ (Eq. 3). Using the same wind speed, the correction factor, A, is 0.665 (Eq. 2). The estimated amount penetrating the cage is then 0.00227 μl/cm² (Concentration$_{\text{inside cage}}$ in Eq. 3). Here, correcting for both collection efficiency and cage penetration results in an exposure underestimate (expressed as percent error) of 78%. This underestimation ranged from 76% (2 m/sec wind speeds) to around 90% (4 m/sec wind speeds).

Dose response relationships (fit to Morgan–Mercer–Flodin sigmoidal models [Morgan et al. 1975]) were determined for both species using both the corrected and uncorrected spray concentration exposure data using CurveExpert (Eq. 4).

$$24\text{-h mortality}(\%) = \frac{\text{ab} + cD^d}{b + D^d} \qquad (4)$$

where D = spray concentration (μl etofenprox/cm²);

a, b, c, d = 0.39, 0.11, 137.7, and 0.22 for *Ae. albopictus,* corrected data;

a, b, c, d = 0.37, 0.09, 151.5, and 0.22 for *Ae. albopictus,* uncorrected data;

a, b, c, d = 22.1, 0.00013, 95.8, and 1.26 for *Cx. quinquefasciatus,* corrected data; and

a, b, c, d = 23.8, 0.00001, 96.8, and 1.20 for *Cx. quinquefasciatus,* corrected data.

Using these relationships, lethal spray concentration levels at which 90% mortality occurred (LC_{90}), for *Ae. albopictus* were determined with LC_{90} values of 0.8 and 0.1 nl/cm^2, for the corrected and uncorrected data, respectively. The LC_{50} values for the *Cx. quinquefasciatus* were 0.54 and 0.06 nl/cm^2 for the corrected and uncorrected data, respectively.

The sampling device and the bioassay cage impact the spray concentration presented to caged mosquitoes through a combination of low sampler collection efficiency and reduced spray penetration into the cage. The degree of exposure is a function of the application rate and spray droplet size, the type of spray collection device used, the type of bioassay cage selected, and the ambient wind speeds present during a spray application (Fritz et al. 2010, 2011a). Although a standardized sampler and/or bioassay cage would increase the comparability between different studies, the effects of wind speed still need to be accounted for. Moreover, the actual exposure levels inside the cages are significantly underestimated (76% to over 90%) if no consideration is given to sampling efficiency and cage filtration. Correcting field-measured data allows not only better comparisons between differing studies, but can also provide better estimates of caged insect mortality versus actual spray concentration exposure levels.

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